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Biological variation of high sensitivity cardiac troponin-T in stable dialysis patients: implications for clinical practice

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Abstract

Background: Changes in high sensitivity cardiac troponin-T (hs-cTnT) concentrations may reflect either acute myocardial injury or biological variation. Distinguishing between these entities is essential to accurate diagnosis, however, the biological variation of hs-cTnT in dialysis

population is currently unknown. We sought to estimate the within- and between-person coefficients of variation of hs-cTnT in stable dialysis patients, and derive the critical difference between measurements needed to exclude biological variation with 99% confidence.

Methods: Fifty-five prevalent haemo- and peritoneal-dialysis patients attending two metropolitan hospitals were assessed on 10 consecutive occasions; weekly for 5 weeks then monthly for 4 months. Assessments were conducted at the same dialysis cycle time-point and entailed hs-cTnT testing, clinical review, electrocardiography, and bio-impedance spectroscopy. Patients were excluded if they developed clinical or physiological instability.

Results: In total 137 weekly and 114 monthly hs-cTnT measurements from 42 stable patients were analysed. Respective between- and within-person coefficients of variation were 83% and 7.9% for weekly measurements, and 79% and 12.6% for monthly measurements. Within-person variation was unaffected by dialysis modality or cardiac co-morbidity. The bidirectional 99% reference change value was –25% and +33% for weekly measurements, and –37% and +58% for monthly measurements.

Conclusions: The between-person variation of hs-cTnT in the dialysis population is markedly greater than within-person variation indicating that hs-cTnT testing is best applied in this population using a relative change strategy. An increase of 33% or a reduction of 25% in serial hs-cTnT concentrations measured at weekly intervals excludes change due to analytical and biological variation alone with 99% confidence.

Keywords: renal dialysis; troponin T; variability.

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Introduction

Acute myocardial injury is a leading cause of hospitalisation and death in the dialysis population (9.8–16 per 100-patient years), and cardiac troponins are among the most frequently requested tests in this group [1–5].

A diagnosis of acute myocardial injury in dialysis patients is contingent on demonstrating a change in serial troponin concentrations in an appropriate clinical context [6, 7]. However, there is currently no evidence-based guidance on how much change in serial measurements discriminates between biological variation and acute myocardial injury leading to considerable diagnostic confusion [8].

Biological or within-person variation describes the random fluctuation of biomarker levels around a homeostatic set-point in healthy individuals or those with stable disease, and is of no clinical significance [9]. Failure to accurately discriminate between changes in troponin concentrations caused by biological variation versus acute myocardial injury can result in unnecessary alarm, inappropriate management, and patient harm [10]. These concerns are particularly relevant following the recent introduction of high sensitivity cardiac troponin T (hs-cTnT) assays which are able to detect small changes in circulating concentrations [11]. Current guidelines either do not specify a magnitude of change in hs-cTnT concentrations that excludes biological variation in dialysis patients [6, 8] or have developed theoretical recommendations based on the analytic performance of assays [7].

The aims of our study were to estimate the within- and between-person variation of hs-cTnT measured at weekly and monthly intervals from a cohort of stable dialysis patients and to use these estimates to calculate the percentage change between serial hs-cTnT measurements needed to exclude biological and analytic variation. We also sought to determine if the within-person variation of hs-cTnT differed according to cardiac co-morbidity or between dialysis modalities.

Materials and methods

Study design and patient recruitment

A prospective cohort study was conducted between October 2010 and April 2012 according to methods described by Fraser and Harris [12]. The study complied with the declaration of Helsinki and received ethics approval from the Metro-South Human Research Ethics Committee (HREC/10/QPAH/131).

Participants were recruited from the in-centre haemodialysis and peritoneal dialysis units of a tertiary-care teaching hospital in Brisbane, and a secondary-care hospital in Logan, Australia. Eligible participants identified from an electronic database of all patients receiving dialysis therapy were adults (aged ≥ 18 -years) on maintenance dialysis for ≥ 90 -days who had a stable dialysis prescription for ≥ 30 -days and a transthoracic echocardiogram ≤ 12 -months prior to screening.

Eligibility criteria were chosen to ensure that the study cohort were physiologically and clinically stable at enrolment, and likely to

remain stable for the duration of the study while still being representative of the dialysis population. Patients were excluded if they had undergone coronary and/or valvular intervention or suffered a myocardial infarction or pulmonary embolism in the 6 months prior to screening; had echocardiographic evidence of severe pulmonary hypertension, severe functional aortic and/or mitral valvular disease, or a left ventricular ejection fraction $< 30\%$; had been hospitalised for any indication in the 30-days prior to screening; had been commenced on or undergone a dose change of a diuretic, β -blocker, aldosterone receptor antagonist, angiotensin converting enzyme inhibitor or angiotensin type-1 receptor blocker in the 30 days prior to screening; had experienced worsening angina, a new cardiac arrhythmia or undergone a dose change of associated therapies in the 30 days prior to screening; had a contraindication to bioimpedance measurement including a pacemaker, implantable cardiac defibrillator, joint replacements, or mechanical heart valve; were pregnant; had advanced malignancy; or were unable to provide informed consent.

Patient assessment

Patients were assessed on 10 consecutive occasions – weekly for 5 weeks then monthly for another 4 months. All assessments were conducted between 6 and 8 AM, prior to the mid-week dialysis session for haemodialysis patients, and between 8 and 10 AM on the same weekday for peritoneal dialysis patients. Patients avoided strenuous exercise prior to assessment.

Several factors affect hs-cTnT concentrations including extracellular volume [13], cardiac rhythm [14], myocardial ischaemia [15], and the dialysis prescription [16, 17]. These influences were assessed using a structured clinical interview, physical examination and medical records review to ascertain interim hospitalisation, changes to medication and/or the dialysis prescription. The Canadian Cardiovascular Society Angina Grading Scale [18] was used to assess change in cardiac ischaemic symptoms and the Truncated Framingham Heart Failure Score [19] was used to assess for evidence of pulmonary oedema at time of review. Patients also underwent a standard 12-lead electrocardiogram and whole body, multi-frequency bioimpedance analysis using the Body Composition Monitor BCM® (Fresenius Medical Care, Asia-Pacific) at each visit to measure intra- and extra-cellular volume. This instrument has a detection limit for change in extracellular volume of 0.87 ± 0.64 L [20, 21].

Specimen collection, storage and analysis

hs-cTnT concentrations were measured at baseline then at weeks 1–4 and months 2–4, providing data for four consecutive weekly and monthly intervals. To ensure that changes in hs-cTnT concentrations during the final measurement interval did not reflect changes in sub-clinical risk, patients were also assessed for stability at week 5 and month 5.

Blood collected in lithium-heparin tubes was centrifuged and plasma separated within 1 h of collection. Plasma was stored at -80°C until assayed. Hs-cTnT has been shown to be stable under these conditions [22].

All samples were batched and analysed together in a single analytic-run in random duplicate by a single expert operator using

a single instrument, and a single batch of reagent, control, and calibrators. Plasma hs-cTnT concentrations (nanograms per litre) were measured on the Cobas e170 instrument using the troponin-T hs kit (Roche Diagnostics, Australia); a monoclonal antibody electrochemiluminescence assay which has a reported detection range of 5–10,000 ng/L. The population 99th centile reference limit for the assay is 14 ng/L [11].

Statistical analysis

Based on a ratio of analytic to within-person variation of <0.5 for hs-cTnT, we estimated that a study sample of 40 patients undergoing hs-cTnT testing on eight occasions over four weekly and monthly intervals would have power =1.0 to estimate the within-person coefficient of variation with a 95% confidence interval of $\pm 1.9\%$ [23]. A sample size of 55 was chosen to allow for dropouts and exclusions due to instability.

Patients were deemed to be unstable if between study visits they underwent a change in dose of diuretic, β -blocker, aldosterone receptor antagonist, angiotensin converting enzyme inhibitor or angiotensin type-1 receptor blocker; a change in severity of cardiac ischaemic symptoms, dose of anti-anginal agents, or cardiac intervention; a change in anti-arrhythmic agent or new cardiac arrhythmia; a change in extracellular volume >1 L on bioimpedance analysis; a change in dialysis modality or prescription; hospitalisation for any reason or exhibited pulmonary oedema defined as a score ≥ 2 on the Truncated Framingham Heart Failure Score [19]. If a study participant was deemed to be unstable, the hs-cTnT concentrations from the intervals preceding and following the event were excluded from the statistical analysis.

Normally distributed variables are summarised as mean $\pm$ standard deviation, and non-normally distributed variables as median and interquartile range. Hs-cTnT concentrations were logarithmically transformed for the variation analyses. We fitted mixed-effects models with random intercepts to calculate the between-person coefficient of variation (CV_b), the within-person coefficient of variation at weekly and monthly intervals (CV_p) and the within-run analytic coefficient variation (CV_A). Outlying variances were excluded using the Reed and Cochran tests, and linear regression analysis was used to exclude participants who demonstrated a consistent trend in hs-cTnT concentrations in either direction which may reflect a change in subclinical risk.

The cohort was also divided into subgroups according to ischaemic heart disease status, severity of left ventricular diastolic dysfunction, presence or absence of left ventricular systolic dysfunction, quartiles of hs-cTnT concentrations at enrolment, and by dialysis modality. CV_p was estimated for each subgroup and compared using Bartlett's test. Ischaemic heart disease was defined as one or more of inducible ischaemia on non-invasive cardiac testing and/or $\geq 50\%$ stenosis in ≥ 1 epicardial coronary artery on coronary angiography and/or a history of myocardial infarction. Left ventricular diastolic dysfunction was graded as absent, mild, moderate or severe according to an established algorithm using echocardiographic measurements [24]. Left ventricular systolic dysfunction was defined as a left ventricular ejection fraction $\leq 50\%$ using Simpson's rule [24].

The index of individuality (IOI) was calculated as CV_p/CV_b . This ratio gives an indication of whether a biomarker is best used within a relative-change monitoring strategy (IOI <0.6) or a reference interval strategy (IOI >0.6). The bidirectional reference change value (RCV)

was calculated according to the method described by Fokkema et al. [25] for logarithmically transformed data as $\exp(-Z \times \sqrt{2} \times \sigma)$ and $\exp(+Z \times \sqrt{2} \times \sigma)$; where $\sigma = \sqrt{\ln(CV_p^2 + 1)}$ and Z is the Z-score of a standard normal distribution corresponding to a given probability.

Results

Patient characteristics

Details of the number of patients assessed, enrolled and included in the final analysis are shown in Figure 1. In total 55 patients were recruited from the haemodialysis ($n=28$) and peritoneal dialysis units ($n=27$) of the participating institutions and their baseline characteristics are summarised in Table 1. Cardiovascular risk factors, including hypertension (100%), diabetes mellitus (40%), and current or former smoking (51%) were highly prevalent. A substantial proportion of the cohort also had evidence of established

Figure 1 Flow diagram of patients assessed for eligibility, enrolled and analysed in the study.

Table 1 Baseline characteristics of the study cohort.

Characteristic	Value, n=55
Male gender, %	45
Age	
Mean±SD, years	59±15
Age distribution, %	
18–49 years	33
50–69 years	40
70–79 years	22
80–90 years	5
Haemodialysis, %	51
Time on dialysis, months	35 (16–58)
Body mass index, kg/m ²	30.2 (28.5–34.6)
Ratio of extracellular to total body water	0.48±0.04
Systolic blood pressure, mmHg	130±15
Diastolic blood pressure, mmHg	74±12
Diabetes mellitus, %	40
Current or former smoker, %	51
Ischaemic heart disease, %	22
Peripheral- and/or cerebro-vascular disease, %	9
Left ventricular ejection fraction	
Mean±SD, %	60±7
Left ventricular systolic dysfunction, %	9
Diastolic dysfunction, %	
Nil	13
Mild	24
Moderate	45
Severe	18
Antihypertensive agents	
Median number of anti-hypertensive agents	1 (1–2)
Proportion on β -blocker, %	42
Proportion on ACE-I or ARB, %	38
hs-cTnT, ng/L	
Median (IQR)	34 (24–150)
Minimum	8
Maximum	241
Proportion of sample (%) with baseline hs-cTnT concentration >14 ng/L	90%

ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin type-1 receptor blocker.

cardiovascular disease including ischaemic heart disease (22%), left ventricular diastolic (87%) and/or systolic (9%) dysfunction, and peripheral- and/or cerebro-vascular disease (9%). Baseline hs-cTnT concentrations demonstrated a right-skewed frequency distribution with a median of 34 (interquartile range 24–54) ng/L. A total of 90% of the study cohort had a hs-cTnT concentration exceeding the 99th centile upper reference limit of the hs-cTnT assay [11].

Weekly and monthly variation of hs-cTnT

Seven patients and their corresponding hs-cTnT measurements were excluded due to hospital admission

(n=3), paroxysmal atrial fibrillation (n=3), and escalating anginal symptoms (n=1). In addition, 36 weekly and 51 monthly hs-cTnT sample-pairs were excluded due to a change in extracellular volume of >1 L between consecutive visits. Hs-cTnT measurements performed over 136 weekly intervals from 42 patients and 113 monthly intervals from 39 patients were included in the final analysis. These data are shown in Figure 2 with excluded measurements represented by gaps.

The respective analytic, within-person, and between-person coefficients of variation of hs-cTnT were 3.1%, 7.9%, and 83% for weekly intervals, and 2.4%, 12.6%, 79% for monthly intervals (Table 2). Between person variation was much greater than within-person variation (Figure 3), yielding low indices of individuality of 0.10 and 0.16 for the weekly and monthly intervals, respectively (Table 2).

Weekly and monthly reference change values for the 80%, 90%, and 99% degrees of statistical confidence are shown in Table 2. Thus, hs-cTnT concentrations measured at weekly intervals needed to increase by 33% or fall by 25% to ensure with 99% confidence that the observed

Figure 2 Variation of high sensitivity cardiac troponin T (hs-cTnT) concentrations measured from each of the stable participants over the weekly and monthly follow-up phases.

Table 2 Estimates of variance components of high sensitivity troponin-T, bidirectional reference change values for stated degrees of statistical confidence and index of individuality over weekly and monthly intervals for stable study participants.

	Coefficient of variation, %			Reference change value			Index of individuality
	Analytic, CV_A	Between-person, CV_G	Within-person, CV_I	99%	90%	80%	$CV_I: CV_G$
Weekly	3.1	83	7.9	−25% and +33%	−17% and +20%	−13% and +15%	0.10
Monthly	2.4	79	12.6	−37% and +58%	−25% and +34%	−20% and +25%	0.16

Figure 3 Box-plot of median (horizontal line), upper and lower quartiles (large rectangle), and range (whiskers) of high sensitivity cardiac troponin T (hs-cTnT) concentrations for each of the stable participants during the weekly follow-up phase.

change exceeded analytic and biological variation alone. Monthly reference change values were larger than weekly values for a given degree of statistical confidence.

The within-person co-efficient of variation did not differ significantly between dialysis modalities, by ischaemic heart disease status, by severity of diastolic dysfunction or across quartiles of hs-cTnT concentration (Table 3). The effect of left ventricular systolic dysfunction on within-person variation was unable to be meaningfully analysed as only one such patient was retained in the final analysis after the exclusion of unstable patients.

Discussion

This study demonstrated that the between-person variation of hs-cTnT across the dialysis population was large and markedly greater than within-person variation for both the weekly and monthly measurement intervals (83% vs. 7.9%, and 79% vs. 12.6%, respectively). This

Table 3 Within-person coefficients of variation of high sensitivity cardiac troponin-T (mean and 95% confidence intervals) by sub-groups of dialysis modality, ischaemic heart disease status, cardiac diastolic function and quartiles of high sensitivity cardiac troponin-T concentration (hs-cTnT).

Sub-group	Weekly within-person coefficient of variation CV_I (95% CI)	p-Value
Dialysis modality		
Peritoneal dialysis	7.5 (6.2–9.0)	0.68
Haemodialysis	8.2 (6.8–10.0)	
Ischaemic heart disease		
Absent	5.7 (4.5–7.3)	0.90
Present	8.5 (7.3–9.9)	
Diastolic dysfunction		
Nil/mild	8.2 (6.8–9.9)	0.78
Moderate/severe	7.7 (6.4–9.3)	
hs-cTnT concentration, ng/L		
1st quartile (8–24)	10.3 (7.8–13.4)	0.31
2nd quartile (25–34)	8.3 (6.5–10.7)	
3rd quartile (35–54)	6.3 (4.6–8.8)	
4th quartile (55–241)	5.6 (4.5–7.1)	

was reflected in the low index of individuality and indicates that hs-cTnT measurements from dialysis patients are best interpreted by comparing serial measurements to each other rather than by comparing single values to a reference interval or a threshold value [26]. These findings support current recommendations on interpreting troponin concentrations measured from dialysis patients [6, 7].

Interpreting serial hs-cTnT concentrations in dialysis patients

We found that the within-person coefficients of variation of hs-cTnT in stable dialysis patients were 7.9% and 12.6% for the weekly and monthly measurement intervals, respectively. Using these values we calculated bidirectional reference change values for pre-specified degrees of

statistical confidence (Table 2) [25]. The weekly 99% reference change value was +33% and -25%, indicating that hs-cTnT concentrations must increase by at least 33% or fall by 25% in the short term to exclude change due to biological and analytic variation alone with 99% certainty. We suggest that this short-term reference change value has the greatest relevance to clinical practice where troponin is measured over hours or days to diagnose acute myocardial injury. In addition, we suggest using the 99% level of statistical confidence as therapies that may be instituted after excluding biological variation carry risks of serious adverse events necessitating a high degree of certainty. It is also important to highlight that acute myocardial injury may result from either coronary or non-coronary aetiologies [6] and that careful clinical assessment remains imperative to determining the underlying cause of acute myocardial injury and to guide its management. We recommend using a relative (e.g., 33%) rather than absolute (e.g., 5–7 ng/L) change criterion as the former has been shown to be diagnostically superior in populations where the majority of individuals have a baseline hs-cTnT concentration exceeding the upper reference limit of the assay used and where the between-person variation of hs-cTnT is large, both of which have been demonstrated in this study [27].

Explicit guidance on the interpretation of serial troponins measured from dialysis patients is necessary as the distinction between troponin fluctuations resulting from biological variation versus those occurring during acute coronary syndromes, particularly non-ST elevation myocardial infarction, is frequently unclear [28, 29]. To date, consensus guidelines on the interpretation of serial troponin measurements in dialysis patients have either provided no guidance on this issue [6, 8] or have based their guidance on the analytic performance of troponin assays, recommending the use of a 20% magnitude of change on the basis that such a Δ equates to three standard deviations of the troponin assay's analytic variation [7]. Based on the within-person coefficient of variation estimated in our study, a 20% change in serial troponin concentrations would only have a 90% degree of statistical confidence for excluding biological and analytic variation, and would therefore not be sufficiently specific for excluding biological variation.

Impact of dialysis therapy and cardiac co-morbidity on biological variation

The weekly and monthly within-person variation of hs-cTnT estimated from dialysis patients in this study was

markedly smaller than corresponding values reported for healthy individuals (15% and 31%, respectively) [30], but similar to those reported for non-dialysis dependent individuals with coronary artery disease (7.3% at 4-hourly intervals and 10.7% at 3-weekly intervals) [31]. These findings are likely explained by the fact that both dialysis patients and non-dialysis dependent patients with coronary artery disease have baseline troponin-T concentrations around or exceeding the 99th centile upper reference limit of the hs-cTnT assay [16, 31] whereas healthy individuals have much lower baseline values [11]. Accordingly, any fluctuations in troponin concentrations caused by biological variation in the former groups represent a smaller proportion of the baseline concentration resulting in a smaller within-person coefficient of variation.

In our study the within-person coefficient of variation of hs-cTnT was not significantly different between dialysis modalities, by ischaemic heart disease status, by severity of diastolic dysfunction, or across quartiles of baseline hs-cTnT concentrations implying that the reference change values described previously can be applied to dialysis patients regardless of their cardiac co-morbidity status or dialysis modality. Furthermore, the analytic variation in our study was consistent with that reported from a multi-centre evaluation of the same analytic method using standard laboratory protocols [11]. It is therefore unlikely that the reference change values reported in this study would be appreciably affected by analytic differences related to 'real world' performance of the hs-cTnT assay.

Strengths and limitations

Our estimates of the within-person variation of hs-cTnT in the dialysis population are supported by the findings of Aakre et al. [32], Pianta et al. [16] and Jacobs et al. [33] who reported a within-person coefficient of variation of 8.3%, 9.7% and 13% for hs-cTnT measured weekly, fortnightly and monthly, respectively, in haemodialysis patients. However, these studies were limited by the exclusion of peritoneal dialysis patients [16, 32, 33], a paucity of repeated measures [16, 33] and most importantly by a lack of rigour in ensuring patient stability, wherein they relied either on patient report [16] or a history of hospitalisation [32, 33] alone to determine stability. The latter introduces the possibility that unstable patients may have been included in the analyses of these studies and impacted the accuracy of their findings. Indeed, Aakre et al. reported that hs-cTnT concentrations measured over 90-min intervals in their study demonstrated a consistent decreasing

trend; this may reflect recovering acute myocardial injury due to haemodialysis-induced cardiac stunning [34] which was not considered by the authors [32].

Our study has a number of important strengths that address these limitations, including the enrolment of both peritoneal- and haemo-dialysis patients, multiple measurements of hs-cTnT, and a rigorous approach towards ensuring stability of all factors affecting hs-cTnT concentrations.

Nevertheless, the present study has several limitations. First, patients with the most severe cardiovascular co-morbidities were excluded to maximise the likelihood that enrolled patients would remain stable for the duration of follow-up. Although this potentially limits the generalisability of the study's findings, it is noteworthy that the baseline characteristics of our study cohort were similar to those reported for prevalent Australasian dialysis patients in the Australian and New Zealand Dialysis and Transplant Registry [35]. Second, the biological variation of hs-cTnT over 3-hourly intervals was not investigated. While this would have most closely approximated the interval over which hs-cTnT is measured in clinical practice, such a study design would have imposed substantial inconvenience on participants, necessitating a 15-h visit and five venepunctures on a non-dialysis day and would likely have limited participation. Finally, our study could not determine if the within-person coefficient of variation of hs-cTnT was affected by left ventricular systolic impairment as only one such patient remained stable during the study.

Conclusions

Based on the findings of this study, it is recommended that hs-cTnT concentrations measured in dialysis patients for the diagnosis of acute myocardial injury be interpreted using a relative change strategy rather than by comparing absolute concentrations to a reference interval. An increase of over 33% or a reduction of 25% in serial hs-cTnT concentrations measured at weekly intervals from dialysis patients excludes change due to biological and analytic variation alone with 99% certainty and should prompt investigation for coronary and/or non-coronary causes of acute myocardial injury; concurrent clinical assessment remains essential to establishing the underlying cause of acute myocardial injury and to guide therapy accordingly. Using the change limits identified in this study, future randomised studies should investigate if early intervention for minor, but significant, changes in cardiac troponin

concentrations improves patient outcomes in the dialysis population as has been demonstrated in the non-dialysis population [36].

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